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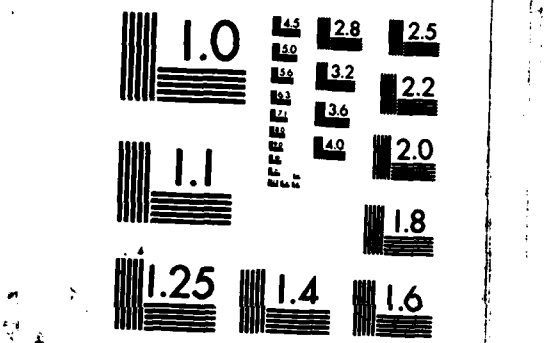

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NONLINEAR PERFORMANCE INTERACTION UPON EXPOSURE TO ANTICHOLINESTERASE AND IONIZING RADIATION

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Final Report for Period January 1983 - April 1983

USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
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NOTICES

This final report was submitted by personnel of the Vulnerability Assessment Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas, under job order 7757-05-58.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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Data on anticholinesterase- and radiation-induced performance deficits suggest that combined exposures would prove more detrimental than either insult presented alone. This hypothesis was tested across radiation (0-7 Gy) and physostigmine (0-0.1 mg/kg) exposure levels and using a rodent rotarod task. The rat was trained to walk on a rotating rod which accelerated from 5 to 45 rpm at 1 rpm/s. The measure of motor performance was the length of time that the animal could stay on the rod. Physostigmine was injected and performance measured at three postirradiation times (45 min and days 4 and 8). The physostigmine-only effect was dose dependent and consistent across test times. The radiation-only effect was also dose dependent, but the performance deficit was also postirradiation-time dependent. The effect of exposure to both radiation and physostigmine was not additive. At 45 min after irradiation, the combined-treatment groups' deficit was greater than either of the single-treatment

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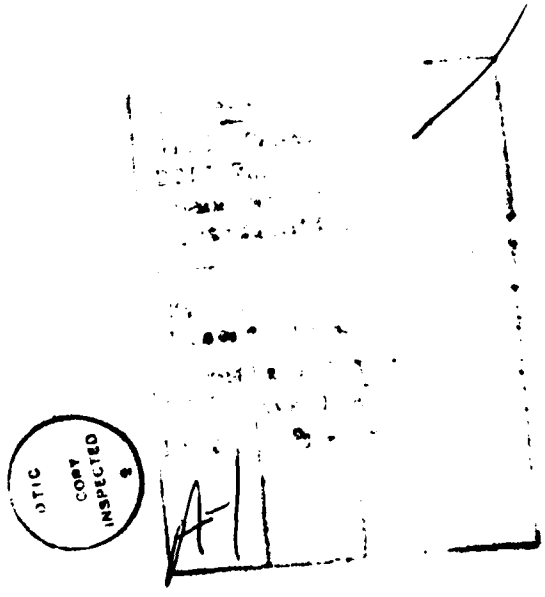
19. ABSTRACT (continued)

groups'. However, by postirradiation day 4, the combined-treatment groups' deficit was no greater than the greatest deficit observed for a single-treatment group. These data are discussed in terms of a possible adaptation mechanism for ionizing radiation as a generalized stressor.



NONLINEAR PERFORMANCE INTERACTION UPON EXPOSURE TO ANTICHOLINESTERASE AND IONIZING RADIATION

INTRODUCTION

Ionizing radiation exposure results in a performance deficit as does exposure to chemical defense agents (anticholinesterases). The two modes of insult involve a number of common mechanisms and biochemical effects; thus, there is reason to believe that a combined exposure could produce a performance deficit greater than exposure to either insult alone.

Rats, mice, guinea pigs, dogs, and rhesus monkeys have been tested for cholinesterase activity after radiation exposure (21, 23). A common finding was a decrease in cholinesterase activity, with a maximum effect 45 min after high radiation exposure (10 Gy, 1 Gy = 100 rads). Williams (23) exposed male rats to gamma radiation and found that whole-blood cholinesterase activity was depressed approximately 17%, particularly 3-10 days after irradiation. The degree of decreased activity was dose dependent up to the highest level tested (6 Gy). Similar findings were also reported by Tominz (20). Blood cholinesterase activity is also decreased upon exposure to anticholinesterase agents such as physostigmine. Krayer et al. (10) reported RBC and serum cholinesterase levels to be dependent on physostigmine dose level; the time course of blood cholinesterase levels in dogs indicates a 30-40% decrease 30 min after an injection of 0.1 mg/kg. A 0.2-mg/kg dose of physostigmine has been shown to reduce mouse blood cholinesterase by 30% (5). Another common effect of anticholinesterase and radiation exposures is decreased motor ability.

Motor ability after X-ray exposure (3-10 Gy) has been described in rats via a swimming time task (9). Although increased performance (sustained swimming) was noted during the first week after exposure, by the second week, swimming ability was greatly reduced. The magnitude of depression was dependent upon the size of the X-ray dose. Surviving animals recovered sufficiently by the ninth postirradiation week to attain their preirradiation performance level. Other studies that have evaluated performance after irradiation (3, 4, 6, 7, 13) have shown motor performance deficit to be a function of level of exposure and postirradiation time. This is also true after physostigmine administration. An anticholinesterase such as physostigmine reduces cholinesterase activity and produces performance decrements in overt behavior, conditioned reflexes, motor ability, and motivation (2, 8). Motor ability has been tested in mice on a rotarod task where they were required to maintain their balance on a 3.2-cm-diameter rod turning at 16.5 rpm (14). An 80% performance decrement was reached between 10 and 15 min after subcutaneous injection of 0.2 mg/kg physostigmine. Similar performance decrements have been reported in rats performing a pole jumping task (17). In this case, 0.25 mg/kg physostigmine produced a maximum effect between 30 and 50 min after injection, with rapid recovery thereafter.

The combined data on anticholinesterase- and radiation-induced cholinesterase reductions suggest that the combined effects of radiation and anticholinesterase exposures may interact and prove more detrimental than either insult presented alone. This hypothesis was tested across various levels of radiation and physostigmine exposure.

METHODS

Experimental Design

Performance measures were evaluated for each test group three times after irradiation. The groups and exposures received are shown in Table 1. Over 450 Sprague-Dawley male rats (225 ± 25 g) were trained and tested. The magnitude of the experiment required that it be divided into two parts. The first part consisted of training, treating, and testing groups 1-4 (22); the second part, performed 1 month later, involved groups 5-11. All animals were maintained on a 12/12 light cycle (0600-1800 light) with free access to food and water. These experiments were performed from 0800-1200 between December 1982 and February 1983.

TABLE 1. TREATMENT GROUPS

Physostigmine (μg/kg)	Radiation Exposure (Gy)*			
	0	2.3	4.7	7.0
0	1,5**	6	7	2
33	8	9		
66	10		11	
100	3			4

*At 0.7 Gy/min.
**Group identification numbers.
Groups 1-4: N=20-22.
Groups 5-11: N=24.

Repeated anticholinesterase injections and testing required the use of an anticholinesterase that was rapidly metabolized; physostigmine was chosen. The effects of physostigmine exposure are pronounced within the first 2 hours after injection, with a rapid return to normal thereafter. Although the physostigmine groups received an injection at each postirradiation test period, the previous physostigmine exposure was not expected to affect the results; i.e., the effects due to a second injection would not be influenced by the residual effects from a previous exposure (14).

Test Sequence

For both parts of the study, the sequence of events was as follows:

a. Rotarod training 3-5 days

b. Radiation exposure or sham (10-min exposure)

c. Physostigmine or placebo (0.9% normal saline) injection (5 min after irradiation)

d. Rotarod testing, 30 min after injection

e. Steps c and d repeated 4 days later

f. Steps c and d repeated 8 days later

The actual exposure day consisted of radiation exposure (or sham) followed 5 min later by physostigmine (or placebo) injection. Animals were confined in Plexiglas tubes and irradiated (or sham irradiated) eight at a time. Each group of eight rats was randomly divided among the appropriate test groups (22). Although all animals were handled in identical fashion during a test day, each test day was unique. Test days 4 and 8, for example, involved no radiation, transportation, or confinement.

Behavioral Training and Testing

The rotarod task provided a measure of motor control (1). The rotarod is a motor-driven 8-cm-diameter rod, with 25-cm-diameter wafers placed perpendicular to the rod to prevent lateral movement. The rat was placed on the stationary rod and oriented with his head in the direction he needed to walk. Timing started when the rod was put into motion. The rod started rotating at 5 rpm. Rotation rate increased at a constant acceleration of 1 rpm/s. When the animal fell (or jumped) from the rod, its weight activated floor-mounted microswitches which stopped the timer, and "on rod" time was recorded. Electric grids beneath the rotating rod produced a footshock (0.1 mA, 1 s duration) when the animal jumped or fell to the grid floor.

Animal training was different between parts 1 and 2 of the study. For part 1, each animal was placed on the rod at least twice per day for 5 training days. With this regimen, only 60% of the animals learned the task. This percentage of trainable animals was consistent with previous reports (1), but reducing the number of training trials to one every other day proved to be just as successful for part 2 of the study. For both parts of the study, the animals with the highest and most consistent training run times were used to make up the test groups.

RESULTS

The results are presented in Table 2 and Figures 1 and 2. Because the experiment was performed in two parts, all data are presented as percent of the appropriate controls. Groups 2-4 are compared to control group 1; groups 6-11 are compared to control group 5. Also, since the extent of handling and confinement was different across test times, scores for each treatment group are relative to scores of the appropriate control group that test day. A complete statistical analysis of these data is presented in Appendix A.

TABLE 2. ROTAROD PERFORMANCE SCORES EXPRESSED AS PERCENTAGE OF CONTROL MEAN (MEAN ± SEM)

Treatment groups	Group No.	Postirradiation test times		
		45 min	4 days	8 days
Controls:	1	100±18	100±23	100±13
	5	100±8	100±10	100±8
Physostigmine only:				
33 µg/kg	8	84±6	90±7	88±7
66 µg/kg	10	74±7	72±8	73±7
100 µg/kg	3	58±11	62±11	62±11
Radiation only:				
2.3 Gy	6	101±8	98±8	97±9
4.7 Gy	7	84±5	78±7	92±8
7.0 Gy	2	69±16	47±9	41±7
Dual-exposure groups:				
33 µg/kg+2.3 Gy	9	81±8	84±9	89±10
66 µg/kg+4.7 Gy	11	57±5	75±7	71±7
100 µg/kg+7.0 Gy	4	43±5	44±7	46±8

SUMMARY OF DATA

1. The physostigmine effect was dose dependent and consistent across test times.

2. The radiation effect was both dose and postirradiation-time dependent (Figure 1). At the lowest exposure level (2.3 Gy), no effect was apparent (Table 1).

3. The physostigmine and radiation dual-exposure effect for groups 4 and 11 are shown in Figure 1. All dual-treatment groups had a greater deficit 45 min after irradiation than either of the corresponding single-treatment groups. By day 4 after irradiation, however, no dual-treatment group had a deficit greater than the largest effect produced by one of the corresponding single-treatment groups.

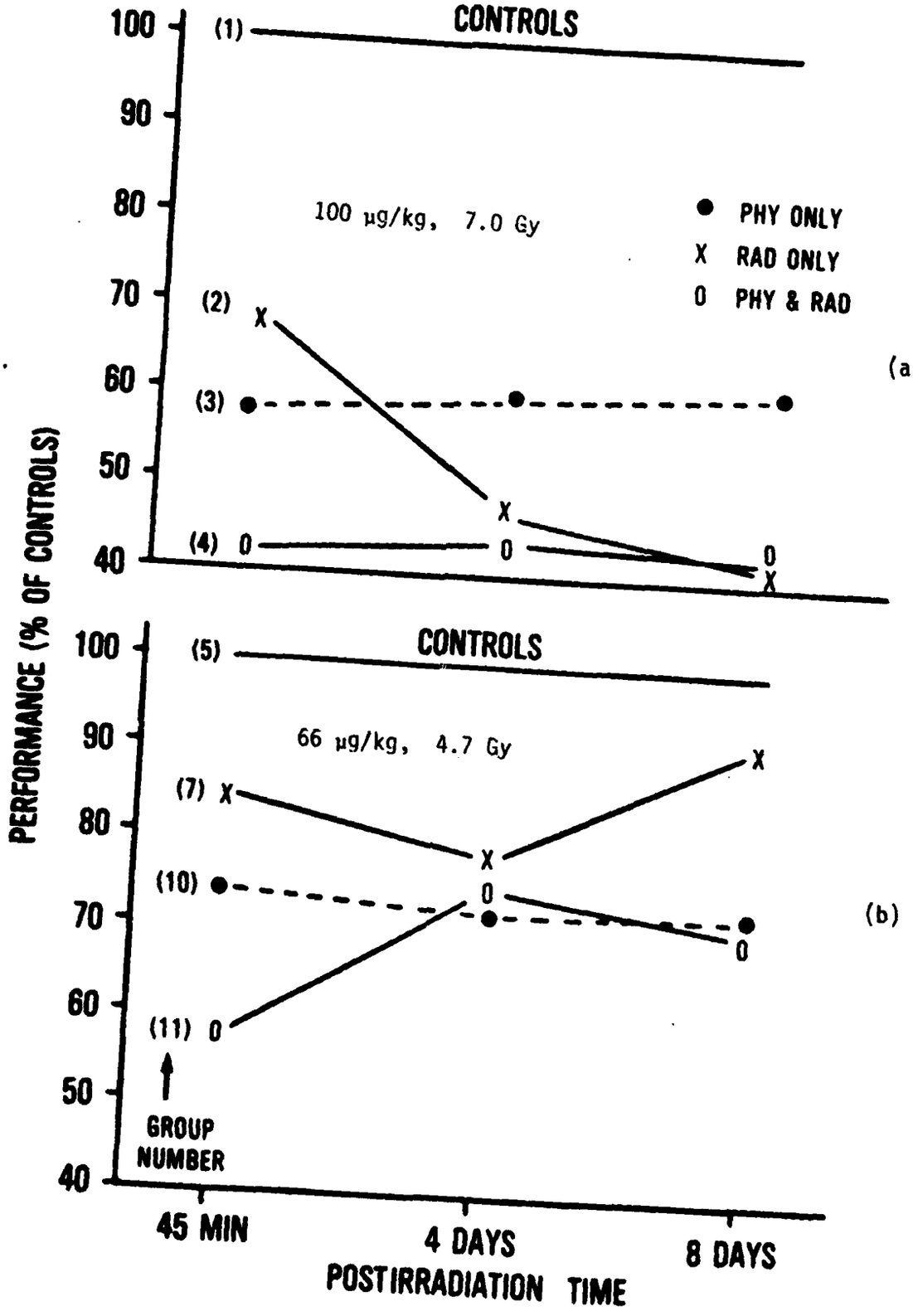


Figure 1. Rotarod performance as a function of postirradiation time. a: High-dose-level groups; b: Middle-dose-level groups.

4. All radiation-exposed groups differed from controls in terms of weight gain (Figure 2). The physostigmine-only groups showed no such significant difference from controls.

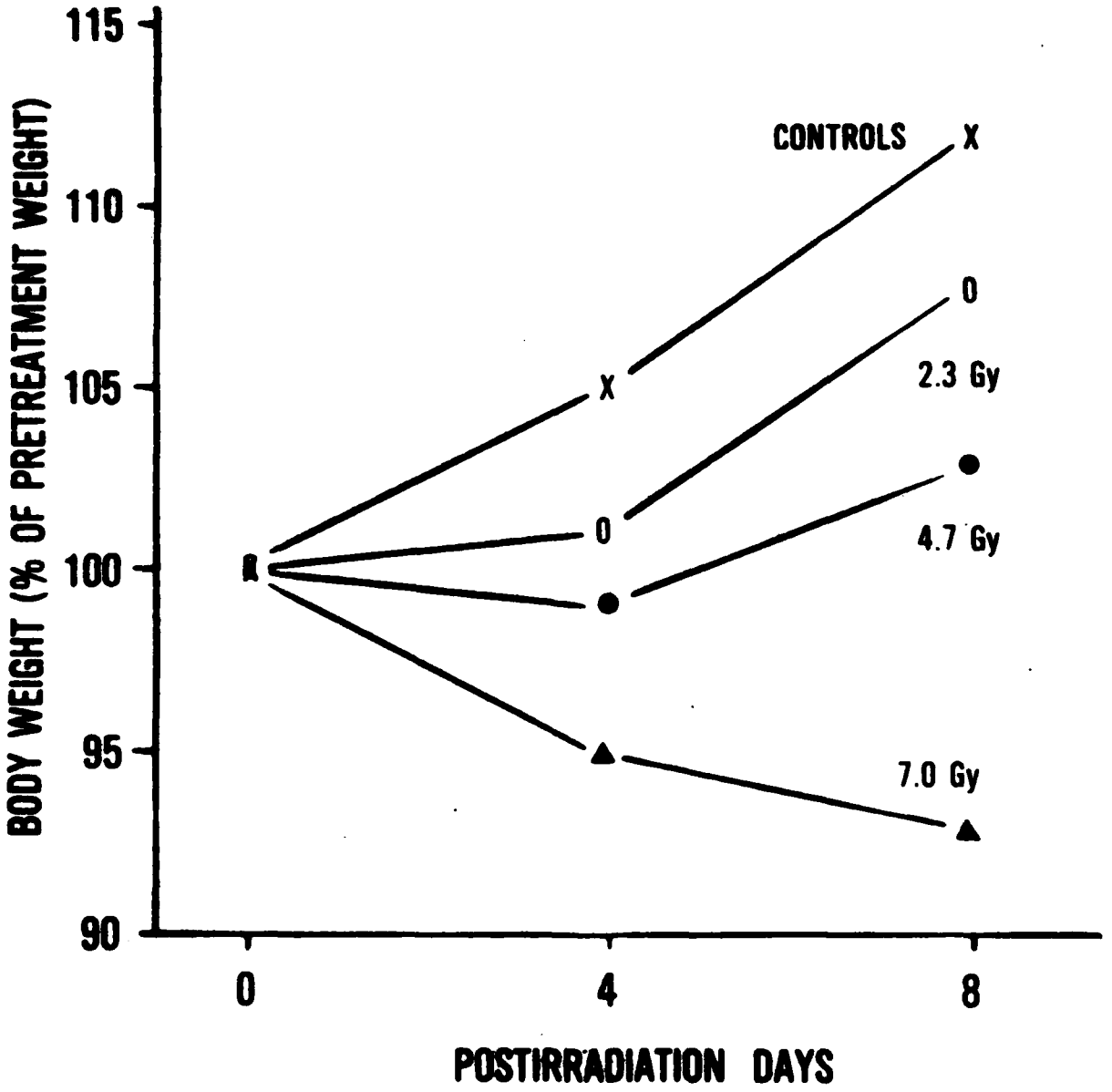


Figure 2. Body weight as a function of radiation and postirradiation time.

DISCUSSION

The hypothesis that combined exposure to ionizing radiation and an anticholinesterase would prove more detrimental than either insult alone was supported by the case where both insults were presented within 15 min time (first test, 45 min after irradiation). The combined effect was apparently linear (additive), which supports the original hypothesis. However, when the anticholinesterase was administered 4 or 8 days after irradiation, no cumulative effect of the two insults was reflected in the performance data. Thus, it seems that the interaction of ionizing radiation and physostigmine is dependent on the length of time between radiation and physostigmine exposures.

A possible reason for a time-dependent mode of interaction can be seen in the radiation-only groups' performance and weight data, which suggest an association between weight gain (general state of health) and performance. The 4.7-Gy group's average weight decreased until 4 days after irradiation and recovered thereafter (Figure 2); the same trend existed for this group's performance (Figure 1b). The 7.0-Gy group experienced a continuing decline in both weight and performance (Figures 1a and 2). These data are consistent with the report that ionizing radiation exposure produces a general stress response (12). Both the physiological and behavioral responses to stress are controlled, via the neuroendocrine system, by the blood immune system which is greatly depressed by ionizing radiation exposure (11). Previous reports (11, 12) and the performance/weight data presented here indicate that the general stress produced by radiation exposure could account for the animals' response to additional stressors.

A well-established phenomenon in medical physiology is that exposure to one stressor reduces the deleterious effects of a second stressor (18, 19). After a stress stimulus, an adaptation (mobilization of the body's resources) provides a period of increased resistance to additional stress (15, 16). One effect of radiation exposure is a temporal reduction in blood cholinesterase (15, 17), so perhaps the adaptation process resulting from radiation exposure involves a resistance to additional decreases in cholinesterase. For postirradiation days 4 and 8, when the performance decrement from 7.0-Gy radiation-only exposure exceeded that due to 100-μg/kg anticholinesterase only, postirradiation injection of physostigmine (corresponding dual-exposure group) did not increase the performance decrement over that produced by radiation only. Only at postirradiation day 8, when the 4.7-Gy radiation-only effect had recovered, was there an apparent effect of adding a physostigmine exposure (4.7 Gy + 66 μg/kg). We conclude that radiation exposure produced a general stress response that counteracted the effects of a second insult, presumably via a resistance to additional changes in cholinesterase levels.

Any mechanistic speculations based on behavioral data must be scrutinized, but such speculation may provide the seed for detailed physiological research. A study designed to evaluate blood or brain cholinesterase levels under the conditions employed here would be most useful.

The data from this study suggest that performance decrements are additive when radiation and anticholinesterase exposures are simultaneous. However, if the anticholinesterase exposure occurs several days after radiation exposure, the resulting deficit of this combined-treatment group is no greater than the greatest deficit observed for a single-treatment group. This suggests that radiation exposure, by whatever mechanism, may produce a resistance, or generalized stress reaction, that effectively counteracts the performance effects of additional stressors.

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APPENDIX A: STATISTICAL ANALYSES OF DATA

The following statistical analyses were used (a reference is given for each):

Paired and unpaired Student's t-tests, the sign test, and Wilcoxon's rank sum test. -- R. G. D. Stell and J. H. Torrie, Principles and Procedures of Statistics. New York: McGraw-Hill Book Company, Inc., 1960.

Levene's F-test for comparing variances. -- H. Levene, "Robust Tests for Equality of Variance," in I. Olkin (ed.), Contributions to Probability and Statistics. Palo Alto, California: Stanford University Press, 1950.

The repeated measurements analysis of variance. -- B. J. Winer, Statistical Principles in Experimental Design, Second Edition. New York: McGraw-Hill Book Company, Inc., 1971.

1. Comparison Between Control Groups (1 and 5): The first question addressed was whether the distributions of rotarod times were the same for the two control groups at the three test times: 45 min, 4 days, and 8 days after irradiation. At each test time the means and/or variances differed between the groups (see Table A-1). In an effort to make parts 1 and 2 of the study more comparable, we decided that for all subsequent analyses, each data value from part 1 of the experiment (groups 2-4) would be reexpressed as a percentage of the corresponding control (group 1) test-time mean; and each data value from part 2, as a percentage of the corresponding control (group 5) test-time mean. Some improvement in comparability was achieved; however, there were still problems. At 45 min after irradiation, for example, the data for group 5 were centered for the most part around 100, with 21 out of 24 values between 50 and 150; whereas for group 1, only 6 out of 21 values were between 50 and 150. Thus the shapes of the distributions for the two control groups were quite different, and in a way that could not be remedied by transforming the data to another set of units. Therefore, analyses that mix data from the two parts of the experiment would seldom meet the assumptions of the analysis; and even if they did, interpreting the test results would likely be difficult. To minimize these problems, the tests subsequently reported were restricted to data from either part 1 or part 2 exclusively.

2. Comparison Between Nonirradiated Groups (1, 5, 8, 10, 3): Groups 5, 8, and 10 were analyzed separately from groups 1 and 3. Each of these two subsets of groups was analyzed in a repeated measurements analysis of variance. The F-tests for group-time interactions were not statistically significant at the .05 level. The F-test for differences between the overall group means was significant ($p = 0101$) for groups 5, 8, and 10 and was close to significance ($p = .0505$) for groups 1 and 3. If the physostigmine administered at each test time had any carryover effect, changes across time in the means for groups 8, 10, or 3 would be expected; however, there were no such changes. Examination of the test results and the group means led to the conclusion that the effect of physostigmine was dose dependent and relatively consistent across test times.

TABLE A-1. SUMMARY STATISTICS FOR CONTROL GROUPS 1 AND 5

Postirradiation test time		Group 1	Group 5	Between-group comparisons: Means	Between-group comparisons: Variances
45 min	N	22	24	$t = 2.17$	$F = 4.91$
	Mean*	15.25	22.21	$df = 44$	$df = 1,44$
	SD	12.61	9.00	$p = .036$	$p = .032$
4 days	N	22	24	$t = 0.04$	$F = 9.02$
	Mean	16.71	16.54	$df = 43$	$df = 1,43$
	SD	17.86	7.86	$p = .97$	$p = .004$
8 days	N	22	24	$t = 2.16$	$F = 0.07$
	Mean	12.23	16.79	$df = 44$	$df = 1,44$
	SD	7.59	6.76	$p = .036$	$p = .79$

* Seconds on the rotarod.

3. Comparisons Between Groups Receiving Radiation but No Drug (1, 5, 6, 7, 2): Groups 5, 6, and 7 were analyzed together and groups 1 and 2 were compared, but the two sets of groups were kept separate for all testing. Initially a repeated measurements analysis of variance was performed for each of these two sets. However, the data were found to violate the assumptions of this analysis, so tests for differences between radiation levels were restricted to comparisons between the groups at the individual times. Table A-2 summarizes the comparisons between groups at the three test times. There is no evidence that group 6 (2.3 Gy) was affected by radiation relative to group 5. Group 7 (4.7 Gy) and group 2 (7.0 Gy) suffered performance decrements, which appeared to last longer for group 2. Thus, it seems reasonable to conclude that the effect of radiation was dependent both on dose level and on time.

4. Comparisons Between Groups Receiving Both Radiation and Drug with Groups Receiving One Insult Only: In testing whether the drug and radiation effects were cumulative, the comparisons of interest are between a group receiving both insults and whichever of the two groups receiving only one of the insults had the greatest performance decrement. Examination of the group means and standard deviations in Table 2 of the report reveals that the only test time for which there was a possible cumulative decrement (statistically) was at 45 min after irradiation. The comparisons of groups 9, 11, and 4 with groups 8, 10, and 3 at 45 min after irradiation are summarized in Table A-3. Group 9 did not differ significantly from group 8, which was expected since the 2.3-Gy radiation level had no detectable effect. Group 11 was statistically significantly ($p = .0228$) smaller than group 10. At the highest drug and radiation level, group 4 was numerically smaller than group 3, but the difference was not significant at the .05 level.

TABLE A-2. SUMMARY STATISTICS FOR NONDRUGGED GROUPS

Postirradiation test time	Groups*	Student's t (df)		1-tail probability: Student's t	1-tail probability: Wilcoxon rank sum test
45 min	5 vs 6	-0.13	(46)	.948	.825
	5 vs 7	1.59	(46)	.0597	.0662
	1 vs 2	1.28	(42)	.1046	.0478
4 days	5 vs 6	0.18	(46)	.430	.443
	5 vs 7	1.88	(46)	.0335	.0348
	1 vs 2	2.15	(41)	.0189	.0252
8 days	5 vs 6	0.24	(46)	.404	.336
	5 vs 7	0.71	(46)	.239	.1821
	1 vs 2	3.90	(42)	.0002	.0002

*Controls = Groups 1 and 5.

TABLE A-3. PERFORMANCE COMPARISONS BETWEEN DRUGGED GROUPS TESTED 45 MIN AFTER RADIATION OR SHAM EXPOSURE

Performance (% of control mean)	Physostigmine: 33 µg/kg + Sham Grp 8	Physostigmine: 33 µg/kg + 2.3 Gy Grp 9	Physostigmine: 66 µg/kg + Sham Grp 10	Physostigmine: 66 µg/kg + 4.7 Gy Grp 11	Physostigmine: 100 µg/kg + Sham Grp 3	Physostigmine: 100 µg/kg + 7.0 Gy Grp 4
$P \leq 16.7$	0	0	1	1	1	3
$16.7 \leq P \leq 33.3$	0	1	2	3	7	8
$33.3 \leq P \leq 50.0$	2	7	1	6	6	4
$50.0 \leq P \leq 66.7$	6	1	6	6	1	1
$66.7 \leq P \leq 83.3$	5	4	6	5	0	5
$83.3 \leq P \leq 100.0$	4	4	3	3	1	1
$100.0 \leq P \leq 116.7$	4	3	2	0	0	0
$116.7 \leq P \leq 133.3$	2	1	2	0	2	0
$133.3 \leq P$	1	3	1	0	2	0
Mean	84.7	81.2	74.1	57.2	58.4	43.1
SD	28.8	38.8	32.6	23.6	49.2	24.4
Student's t-test						
t(df)	0.25(46)		2.05(46)		1.29(40)	
1-tail prob	.4028		.0228		.1015	
Wilcoxon rank sum test						
1-tail prob (approx.)	.2889		.0233		.2563	